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Test Plan for Sulfanilic acid (CAS No. 121-57-3) and o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid) (CAS No. 6471-78-3)

Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

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.

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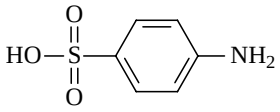
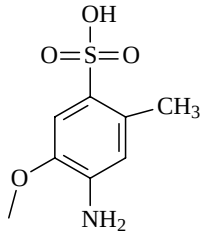
1 IDENTITY OF SUBSTANCES



Sulfanilic acid
CAS No. 121-57-3



o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid)

CAS No. 6471-78-9

2 CATEGORY ANALYSIS

2.1 INTRODUCTION

The International Association of Color Manufacturers (IACM) has volunteered to participate in the EPA's Chemical "Right-to-Know" Program. IACM is committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and, where needed, conducting additional testing on the chemicals used by the color industry in order to assure their human and environmental safety. The category analysis, test plan, and robust summaries presented represent the first phase of IACM's commitment to the Chemical "Right-to-Know" Program.

2.2 BACKGROUND INFORMATION

This category analysis and test plan provides data for sulfanilic acid (CAS No. 121-57-3) and for the related substance, o-toluene sulfonic acid, 4-amino-5-methoxy- (p-cresidine sulfonic acid) (CAS No. 6471-78-3). Both are used as intermediates in the production of azo dyes. In anaerobic conditions, azo dyes undergo bacterial azo reduction in the gastrointestinal tract of rats, rabbits and humans to yield the corresponding sulfonated aromatic amines, such as sulfanilic acid and p-cresidine sulfonic acid (Allan & Roxon, 1974; Chung et al., 1978; Dubin & Wright, 1975; Roxon et al., 1967a; Roxon et al., 1967b; and Watabe et al., 1980). These data support the conclusion that human health data on FD&C Yellow 5 and 6, and FD&C Red 40 is relevant to sulfanilic acid and p-cresidine sulfonic acid in that FD&C Yellow 5 and 6 and FD&C Red 40 form sulfanilic acid and p-cresidine sulfonic acid, respectively, in animals, which is then absorbed, metabolized and excreted.

2.3 STRUCTURAL CLASSIFICATION

Sulfanilic acid and p-cresidine sulfonic acid are both aromatic aminosulphonic acids. p-Cresidine sulfonic acid is sulfanilic acid substituted with *o*-methoxy, and a *m*-methyl groups.

2.4 PHARMACOKINETICS AND METABOLISM

Sulfanilic acid is principally excreted unchanged in the urine and in the feces with lesser amounts excreted as the N-acetyl derivatives of the amine functional group. N-acetylation occurs both in the gastrointestinal tract and following absorption as shown by N-acetyl conjugates appearing in the urine and in the feces.

Following oral administration of 25 mg of sulfanilic acid to rats, the 24-hr urine showed approximately 40% excreted unchanged in the urine with approximately 15% excreted in the conjugated form which was presumed to be the N-acetyl derivative. Approximately 40% (30 % excreted unchanged and 10% excreted in the conjugated form) was excreted in the feces by 48 hours (Scheline and Longberg, 1965).

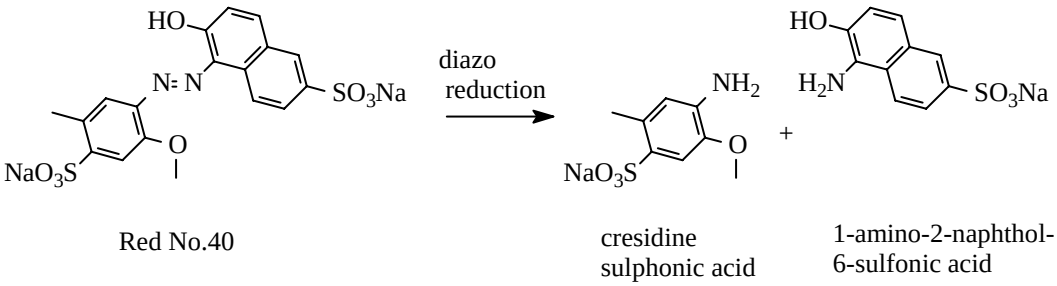
In a study comparing sulfanilic acid in the rat, rabbit and guinea-pig, 10 mg/animal was administered via gavage to the rats (10 animals) and guinea-pigs (10 animals), while 120 mg/animal was administered to the rabbit (8 animals). The remaining two rabbits received 630 mg/kg bw via gavage. All three animals excreted sulfanilic acid in the urine as either the free material or as an N-acetyl derivative. The rat excreted 36 and 13%, the guinea-pig 12 and 36% and the rabbit 76 and 16% as the unchanged material and metabolite, respectively. The authors reported 26, 74 and 17 % as the percentage of acetylated compound in the recovered material (McMahon and Reilly, 1969).

Azo dyes undergo bacterial azo reduction in the anaerobic environment of the gastrointestinal tract of rats, rabbits and humans to the corresponding sulfonated aromatic amines, such as sulfanilic acid and p-cresidine sulfonic acid. Studies across species have shown that FD&C Yellow 5 is reduced to sulfanilic acid and a pyrazalone metabolite, which is further reduced by intestinal bacteria first to p-phenylhydrazinesulfonic acid and then to sulfanilic acid; FD&C Yellow 6 is reduced to sulfanilic acid and amino-2-naphthol-6-sulfonic acid; and FD&C Red 40 is reduced to p-cresidine sulfanilic acid and amino-2-naphthol-6-sulfonic acid in the intestines (see Figure 1) (Allan & Roxon, 1974;

Chung et al., 1978; Dubin & Wright, 1975; Hazleton Laboratories, 1975b; Honohan *et al*., 1977; Roxon et al., 1967a; Roxon et al., 1967b; and Watabe et al., 1980).

Figure 1. Reduction of azo dyes

Metabolism of FD&C Red 40

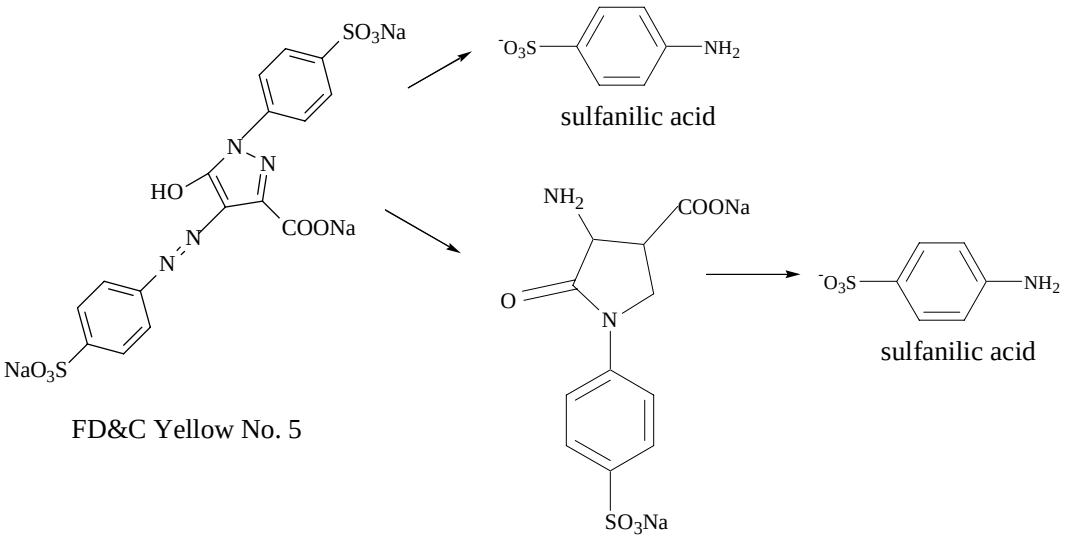


Red No.40

cresidine sulphonic acid

1-amino-2-naphthol-6-sulfonic acid

Metabolism of FD&C Yellow 5



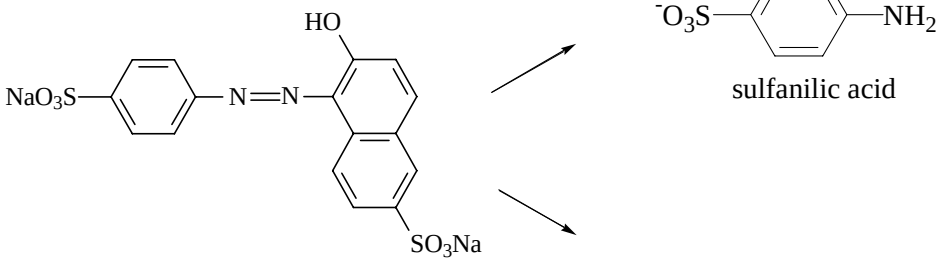
FD&C Yellow No. 5

sulfanilic acid

sulfanilic acid

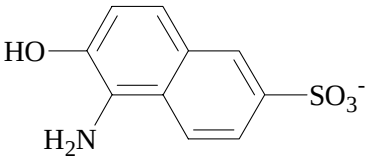
4-amino-5-oxo-1(p-sulphophenyl)-2-pyrazoline-3-carboxylic acid

Metabolism of FD&C Yellow 6



FD&C Yellow No. 6

sulfanilic acid



amino-2-naphthol-6-sulfonic acid

3 TEST PLAN

3.1 CHEMICAL AND PHYSICAL PROPERTIES

3.1.1 Melting Point

Sulfanilic acid decomposed without melting when heated to 288 °C (Merck, 1997). p-Cresidine sulfonic acid has a calculated melting point of 152.63 °C (MPBPVPWIN EPI Suite, 2000).

3.1.2 Boiling Point

The boiling point of sulfanilic acid was calculated to be 363.26 °C while the boiling point of p-cresidine sulfonic acid was calculated to be 398.51 °C (MPBPVPWIN EPI Suite, 2000).

3.1.3 Vapor Pressure

The calculated vapor pressure for sulfanilic acid has been reported to be 2.62 X 10^{-9} mm Hg at 25°C while the vapor pressure of p-cresidine sulfonic acid is calculated to be 1.02 X 10^{-8} mm Hg at 25°C (MPBPVPWIN EPI Suite, 2000). Based on the presence of polar sulfonic acid and amine functional groups, a very low vapor pressure is anticipated for these substances.

3.1.4 Octanol/Water Partition Coefficients

The experimental log K_{OW} value for sulfanilic acid is –2.16 (Okamoto et al., 1991). The calculated log K_{OW} value closely matches the experimental value for sulfanilic acid and is -2.08 (KOWWIN EPI Suite, 2000). An experimental value is not available for p-cresidine sulfonic acid, however the calculated value is -1.45 (KOWWIN

EPI Suite, 2000). Given the presence of sulfonic acid and amine functional groups on both sulfanilic acid and p-cresidine sulfonic acid, similar log K_{OW} values are anticipated.

3.1.5 Water Solubility

Sulfanilic acid has a reported water solubility of 10,800 mg/L at 20 °C (Yalkowsky and Dannenfelser, 1992). The calculated value for sulfanilic acid is 41,530 mg/L at 25 °C (WSKOW EPI Suite, 2000). An experimental value is not available for p-cresidine sulfonic acid, however the calculated value is 6208 mg/L at 25 °C based on the calculated log K_{OW} (WSKOW EPI Suite, 2000). The calculated values for both sulfonic acid derivatives are consistent with the experimental value available for sulfanilic acid.

3.1.6 New Testing Required

None.

3.2 ENVIRONMENTAL FATE AND PATHWAYS

3.2.1 Photodegradation

The calculated half-life for hydroxyl radical reactions is 5.5 and 2.4 hours for sulfanilic acid and p-cresidine sulfonic acid, respectively (AOPWIN EPI Suite, 2000). These calculated short half-lives are consistent with ready abstraction of the sulfonic acid hydrogen by hydroxyl radicals.

3.2.2 Stability in Water

Potential reactivity in water would involve desulfonation of the aromatic sulfonic acid. In aqueous acid (sulfuric acid), aromatic sulfonic acids desulfonate at high temperatures (i.e. 100 to 175 ^{o}C). These conditions would not typically be encountered in the environment. Therefore, sulfanilic acid and p-cresidine sulfonic acid are anticipated to be stable in water.

3.2.3 Biodegradation

In a study assessing biodegradability by manometric respirometry conducted by the Commission of European Communities, five of the eight laboratories employed in the study determined sulfanilic acid was <20% biodegradable. A sixth laboratory where the inoculum was adapted to sulphonic acids found sulfanilic acid to be 70% biodegradable at 10 days and 90% biodegradable at 28 days. A seventh laboratory found duplicates on one occasion to give no removal while a single determination yielded 12% degradation. The eighth laboratory reported 6, 14 and 62% biodegradation after 28 days in three triplicates (Commission of European Communities, 1983). Based on results of the majority of eight separate laboratory determinations, it can be concluded that both sulfonic acid derivatives are not readily biodegradable.

Sulfanilic acid and p-cresidine sulphonic acid were predicted not readily degradable by BIOWIN model calculations (AOPWIN EPI Suite, 2000).

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70 (EPIWIN EPI Suite, 2000). The principal input parameters into the model are molecular weight, melting point, vapor pressure, water solubility, and log K_{OW}.

As expected, the model predicts that sulfanilic acid and p-cresidine sulfonic acid are distributed completely to the water and soil compartments. These data are consistent with experimental ecotoxicity data for aromatic sulfonic acid derivatives that demonstrate essentially no absorption and toxicity to fish even at concentrations exceeding 1000 mg/L (Alstoffe, 1992).

3.2.5 New Testing Required

None.

3.3 ECOTOXICITY

3.3.1 Acute Toxicity to Fish

Extensive studies on the ecotoxicity of benzene sulfonic acids have been conducted and indicate a low order of toxicity to fish (Alstoffe, 1992). An experimental 96-hour LC50 value is available for sulfanilic acid and was reported to be 100.4 mg/L (Alstoffe, 1992). Based on input parameters for molecular weight, water solubility, and melting point, the calculated 96-hour LC50s for sulfanilic acid and p-cresidine sulfonic acid are 5.39 X 10^5 and 2.31 X 10^5 mg/L, respectively (ECOSAR EPI Suite, 2000) indicating a very low order of acute toxicity to fish. Based on the slight increase in K_{OW} (-1.45) for p-cresidine sulfonic acid compared to that of sulfonic acid (-2.08) and the small difference in calculated 96-hour LC50 values, a 96-hour LC50 value slightly less than 100 mg/L is expected for p-cresidine sulfonic acid.

3.3.2 Acute Toxicity to Aquatic Invertebrates

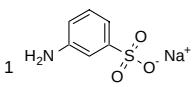
An experimental 24-hour EC50 value with *Daphnia* is available for sulfanilic acid and was reported to be 109.13 mg/L (Alstoffe, 1992). The calculated 48-hour LC50 values for sulfanilic acid and p-cresidine sulfonic acid in daphnids (151 and 128 mg/L, respectively) based on input parameters for molecular weight, water solubility, and melting point (ECOSAR EPI Suite, 2000) also indicating a low order of acute toxicity. These values are consistent with the experimental value available for sulfanilic acid. Based on the consistency in the measured and calculated values, the EC50 of both substances is concluded to be >100 mg/L.

3.3.3 Acute Toxicity to Aquatic Plants

Experimental values are available for the closely related substance, 3-amino-benzenesulfonic acid, monosodium salt[1]. The 72-hr EC50 for this substance was reported to be >500 mg/L in algae (Alstoffe, 1992). Calculated EC50's are not available. The calculated chronic toxicity value (ChV) for 3-amino-benzenesulfonic acid, monosodium salt, to green algae is 14,287 mg/L (log Kow = -3.87). Similarly, the calculated chronic toxicity value for sulfanilic acid is 11,234 mg/L to green algae while the chronic toxicity value for p-cresidine sulfonic acid is 6005 mg/L based on input parameters for molecular weight, water solubility, and melting point (ECOSAR EPI Suite, 2000)

3.3.4 New Testing Required

None.

1 

3-amino-benzenesulfonic acid, monosodium salt

3.4 HUMAN HEALTH TOXICITY

In anaerobic conditions, azo dyes undergo bacterial azo reduction in the gastrointestinal tract of rats, rabbits and humans to the corresponding sulfonated aromatic amines, such as sulfanilic acid and p-cresidine sulfonic acid. Studies across species have shown that FD&C Yellow 5 is reduced to sulfanilic acid and a pyrazalone metabolite, which is further reduced by intestinal bacteria first to p-phenylhydrazinesulfonic acid and then to sulfanilic acid; FD&C Yellow 6 is reduced to sulfanilic acid and amino-2-naphthol-6-sulfonic acid; and FD&C Red 40 is reduced to p-cresidine sulfanilic acid and amino-2-naphthol-6-sulfonic acid in the intestines (see Figures) (Allan & Roxon, 1974; Chung et al., 1978; Dubin & Wright, 1975; Hazleton Laboratories, 1975b; Honohan *et al*., 1977; Roxon et al., 1967a; Roxon et al., 1967b; and Watabe et al., 1980).

Few human health toxicity studies have been carried out on aromatic amino sulfonic acids (AASA) such as the two considered here most likely due to the expected low toxicity of these materials. Jung et al. has proposed that it is reasonable to consider the carcinogenicity data available on the dyes when reviewing the safety of aromatic amino sulfonic acids (Jung et al., 1992) given that hypothetically the dyes can only form AASAs on reductive cleavage of the azo group. In the case of FD&C Red 40, and FD&C Yellow 5 and 6, numerous human health studies are available and have been submitted and deemed adequate for fulfilling the HPV endpoints by the EPA. Given the very high doses employed in these tests, substantial amounts of either sulfanilic acid or p-cresidine sulfonic acid are presumably formed in the gastrointestinal tract during these assays.

3.4.1 Acute Toxicity

Acute toxicity data are available for the parent compounds.

FD&C Yellow No. 5

In reports submitted to the World Health Organization, the acute oral LD50 in mice was reported to be 12,750 mg/kg bw for FD&C Yellow No. 5 (National Institute of Hygienic Sciences of Japan, 1964). In rats, the LD50 for FD&C Yellow No. 5 by intraperitoneal injection was reported to be 2,000 mg/kg bw and the LD50 by intravenous injection was reported to be 1,000 mg/kg bw (Deutsche Forschungsgemeinschaft, 1957).

FD&C Yellow No. 6

The low acute oral toxicity of FD&C Yellow No. 6 is reflected by LD50 values greater than 2,000 mg/kg (Lu and Lavalle, 1964) and 10,000 mg/kg (Gaunt *et al*., 1967) in rats, and greater than 6,000 mg/kg in mice (Gaunt *et al*., 1967).

In a pre-GLP acute toxicity study, adult male Wistar rats were administered 2000 mg/kg bw of FD&C Yellow No. 6 *via* stomach tube. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 2000 mg/kg bw (Lu and Lavalle, 1964).

In another pre-GLP acute toxicity study, groups of five male and female rats each were administered FD&C Yellow No. 6 in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. No deaths at up to 10,000 mg/kg bw. Slight diarrhea reported for 24 hours following treatment. Feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 2000 mg/kg bw (Gaunt *et al*., 1967).

Groups of five male and female mice each (body weights: 20-25 kg) were administered FD&C Yellow No. 6 in aqueous solution. Animals were fasted for 18 hours

prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. No deaths at up to 6000 mg/kg bw Slight diarrhea reported for 24 hours following treatment. Feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 in mice was determined to be greater than 6000 mg/kg bw (Gaunt *et al*., 1967).

Groups of five male and female rats each (body weights: males 200-250 g; females 150-200 g) were administered FD&C Yellow No. 6 in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. Slight diarrhea reported for 24 hours following treatment. Skin, feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 3800 mg/kg bw (Gaunt *et al*., 1967).

Groups of five male and female mice each (body weights: 20-25 kg) were administered FD&C Yellow No. 6 in aqueous solution. Animals were fasted for 18 hours prior to treatment and observed for 7 days following treatment. Necropsies were performed on animals that died and some survivors. Slight diarrhea reported for 24 hours following treatment. Skin, feces and urine were colored orange. No macroscopic changes reported upon necropsy. The oral LD50 for FD&C Yellow No. 6 was determined to be greater than 5500 mg/kg bw (Gaunt *et al.*, 1967).

FD&C Red No. 40

Pre-GLP acute toxicity studies were conducted on FD&C Red No. 40 in rats and dogs. Six groups of five male and five female Sprague-Dawley rats were each administered the test substance in a 10% weight/volume solution. The dosage levels tested were 215, 464, 1,000, 2,150, 4640, and 10,000 mg/kg bw. Observations were made immediately following dosing, at 1, 4, 24, 48-hours and once daily thereafter up to 14 days. Following the observation period, the animals were weighed, sacrificed by cerebral

concussion and necropsied. Clinical observations were normal with the exception of red-colored feces in both sexes at all dose levels and red-colored urine at the three highest dose levels in the female animals. There were no deaths at any dose level tested. The acute LD50 was determined to be greater than 10,000 mg/kg bw/day for adult male and female Sprague-Dawley albino rats administered FD&C Red No. 40 *via* gavage (Hazelton Laboratories, Inc., 1965a).

Two male Mongrel dogs were administered FD&C Red No. 40 in an aqueous solution at a dose level of 5,000 mg/kg bw. Observations were made immediately following dosing and daily thereafter for 7 days. Following the observation period, the animals were weighed, sacrificed and necropsied. Red diarrhea was observed 30 minutes following dosing in one animal, which was followed by emesis. Red urine was reported for the other animal. Red stools were reported for both dogs one day following dosing. From the third day until the seventh day, both animals appeared normal with respect to appearance, behavior, appetite and elimination. Gross necropsy revealed fibrotic changes and decreased weight in a kidney of one test animal. This finding was not considered treatment related, but was rather considered to be a chronic lesion. The spleen also appeared enlarged in this test animal. In the other test animal, hookworms were observed in the gastrointestinal tract. There were no deaths at the dose level tested (5,000 mg/kg bw). The acute LD50 was determined to be greater than 5,000 mg/kg bw/day for male Mongrel dogs administered FD&C Red No. 40 *via* gavage (Hazelton Laboratories, Inc. 1965b).

3.4.2 *In vitro* and *In vivo* Genotoxicity

3.4.2.1 In vitro

Sulfanilic acid tested negative in several reverse mutation assays using *Salmonella typhimurium* TA1538, TA1535, TA 97, TA98, TA100 up to 10,000 micrograms/plate with and without metabolic activation (Chung et al., 1978; Zeiger, 1988; Litton Bionetics, 1985). Negative assay results were also reported in a GLP sister chromatid exchange assay (SCE) when sulfanilic acid was incubated with Chinese

Hamster Ovary cells with and without metabolic activation up to 5.0 mg/ml (Litton Bionetics, 1985). Sulfanilic acid was considered inactive in a GLP mouse lymphoma forward mutation assay with and without metabolic activation at concentrations up to 5000 micrograms/ml (Litton Bionetics, 1985).

3.4.2.2 In vivo

In vivo genotoxicity data are available for the parent compound, FD&C Yellow 5 and Yellow No. 6. In an *in vivo* UDS assay, six to eight male Sprague-Dawley rats weighing 200-300 g were administered 500 mg/kg bw. FD&C Yellow No. 5 *via* gavage. FD&C Yellow No. 5 did not induce unscheduled DNA synthesis at the dose level tested (Kornbrust and Barfknecht, 1985). FD&C Yellow No. 6 tested negative in the rat micronucleus test at a single dose level of up to 1,000 mg/kg bw/day (Westmoreland and Gatehouse, 1991).

3.4.3 Repeat Dose Toxicity

FD&C Yellow No. 5

Groups of sixty male and sixty female mice each were administered 0, 0, 0.5, 1.5 or 5.0% FD&C Yellow No. 5 in the diet daily for 104 weeks. Animals were housed individually and fed the test diet *ad libitum*. Clinical observations were recorded twice daily, while detailed physical examinations and palpation for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for weeks 16-26 and monthly from week 26 until the end of the study. The intake of the test substance was determined from body weight, food consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (5.0%) and any animals with gross lesions or masses.

Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes, stomach, thymus, thyroid gland including parathyroid, trachea, and urinary bladder. Physical observations included hair loss, lacrimation, nasal discharge, staining of hair in the anogenital region and soft stools. None of these observations was attributed to administration of the test substance. Discolored urine and feces was reported at all treatment levels within one week of the study initiation. Mean body weights of both sexes were slightly lower than controls at the 5.0% treatment group for a number of sampling intervals, and male mice at the 1.5% treatment group were lower than controls for a number of sampling intervals. These differences were significantly lower in some intervals. Mean food consumption was significantly increased in male mice at the 5.0% treatment level. No statistically significant differences were reported for any of the hematological parameters. Common neoplastic, inflammatory, and degenerative lesions were reported amongst treated and control animals but were not considered to be treatment related. The decrease in mean body weight at the 5.0% treatment level was not considered toxicologically significant give the non-nutritive character of FD&C Yellow No. 5. The no observable adverse effect level (NOAEL) of 5.0% providing an average daily intake of 8103 or 9753 mg/kg/day was established for male and female mice under the conditions of this study (Borzelleca and Hallagan, 1988b).

In a lifetime toxicity/carcinogenicity study, groups of rats (60/sex/group) were administered 0, 0, 0.1, 1.0 or 2.0% FD&C Yellow No. 5 in the diet daily for approximately 2 months prior to mating. In the high-dose study, 60/sex/group received 0 or 5% FD&C Yellow 5 for approximately 2 months prior to mating. The 3 controls groups received the basal diet only. A maximum of 2 rats/sex/litter were randomly selected for the chronic phase of the study. There were 70/sex/group at the initiation of the chronic phase and these offspring were exposed to the same dietary levels as their parents for 113 weeks. Animals were housed individually and fed the test diet *ad libitum*.

Clinical observations were recorded twice daily with at least 5 hours between observations. Detailed physical examinations and palpation for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for the next 12 weeks and every 4 weeks thereafter until the end of the study. The intake of FD&C Yellow 5 was determined from body weight, food consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on ten randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (2.0 or 5.0%) from each study and also on 10 rats randomly selected from each group for an interim sacrifice at 12 months. Histology was also performed on any animal with gross lesions or masses. Tissues examined included adrenal glands, aorta, blood smear, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, duodenum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes with epididymides, stomach, thymus, thyroid gland including parathyroid, trachea, urinary bladder, uterus.

During the *in utero* phase, there were no treatment-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or number of live and still-born pups. Slight decreases in body weight (4-5%) and slight increases in food consumption were noted in the F0 rats treated at dietary level of 5.0%. Two F0 female controls rats died during the *in utero* phase of the original study and one male and one female from the control and 5.0% group, respectively, died during the *in utero* phases of the high-dose study. There were no treatment related effects on pup survival.

In the F1 generation, a yellow tint was reported at all intake levels above 0.1%. At the 1.0% dietary level, group mean body weights at termination for both sexes were lower than the control animals, but the difference was only statistically significant for the

females. In the high dose study (5.0% dietary level), group mean body weights were significantly lower in both sexes at termination. Food consumption was similar for control and treated animals at the 0.01, 1 or 2% dietary levels, but was slightly higher at the 5% level in the high-dose study, although not statistically significant. Hematological, clinical chemistry and urinalysis parameters did not differ significantly from the controls. Necropsies at one year did not reveal any treatment-related gross or microscopic changes. At study termination, no treatment-related effects were reported on survival. No treatment-related changes were reported at gross necropsy. Histological evaluation revealed a variety of lesions, including neoplasms, present at similar incidences in control and treated animals. The authors considered the lesions to be spontaneous and not related to administration of the test material. The decrease in mean body weight at the 5.0% treatment level was not considered toxicologically significant give the non-nutritive character of FD&C Yellow No. 5. A NOAEL of 5.0% providing an average daily intake of 2641 mg/kg/day and 3348 mg/kg/day for male and female rats, respectively, was reported under the conditions of this study (Borzelleca and Hallagan, 1988a).

FD&C Yellow No. 6

Groups of ten male and ten female mice each were administered 0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm FD&C Yellow No. 6 in the diet daily for 12 weeks followed by one week of control diet only. Animals were housed five per cage and fed the test diet *ad libitum.* The animals were observed twice per day and weighed weekly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Mean body weight gain was decreased compared to controls among male mice receiving the 100,000 ppm intake level. Decreases in body weight gain were also reported for female mice at all intake levels, and was dose related from 12,500 ppm to 100,000 ppm. Gross and histopathological examinations revealed no treatment related lesions in male or female mice at any intake level. The NOAEL's were reported to be 50,000 ppm and less than 6,000 ppm for male and female mice, respectively (NTP, 1981).

Groups of ten male and ten female rats each were administered 0, 6000, 12,500, 25,000, 50,000 or 100,000 ppm FD & C Yellow No. 6 in the diet daily for 12 weeks followed by one week of control diet only. Animals were housed five per cage and fed the test diet *ad libitum.* The animals were observed twice per day and weighed weekly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. No animals died during the study. Decreases in mean body weight gain were reported for male rats at the 25,000, 50,000 or 100,000 ppm intake levels. For female rats, decreases in mean body weight gain were reported at the 12,500, 25,000, 50,000 or 100,000 ppm intake levels. Bone marrow hyperplasia was reported in all examined animals at the 50,000 or 100,000 ppm intake levels. The NOAEL's were reported to be 6000 ppm for female rats and 12,500 ppm for male rats (NTP, 1981).

Groups of fifty male and fifty female mice each were administered 12,500 or 50,000 ppm FD&C Yellow No. 6 in the diet daily for 103 weeks. Fifty male and female mice each served as concurrent controls. Animals were housed five per cage and fed the test diet *ad libitum*. The animals were observed twice per day and weighed at least monthly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, lymph nodes, pancreas, parathyroids, pituitary gland, rectum, skin, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder. The mean body weights of male and female mice administered the high dose were slightly lower than the control animals throughout most of the study. The survival of male and female mice was similar between treated animals and controls (males: control 38/50 (76%); low dose 40/50 (80%); and high dose 33/50 (66%) and females: control 38/50 (76%); low dose 35/50 (70%) and high dose 43/50 (86%)). An increased incidence in hepatocellular carcinomas was reported among males in the low (46%) and high (32%) dose groups compared to the control males (26%), but was only a significant difference in the low dose mice. No significant differences were observed in the female animals. The increased incidence in hepatocellular carcinomas reported for male mice was not

considered clearly related to administration of the test material given the variability in tumor occurrence in control male B6C3F1 mice and because the incidence of these tumors was not significantly increased in the high dose male mice. The authors reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of FD&C Yellow No. 6 in B6C3F1 mice (NTP, 1981).

Groups of fifty male and fifty female rats each were administered 12,500 or 50,000 ppm FD & C Yellow No. 6 in the diet daily for 103 weeks. Ninety male and female rats each served as concurrent controls. Animals were housed five per cage and fed the test diet *ad libitum*. The animals were observed twice per day and weighed at least monthly. Necropsies were performed on all animals. Gross and histopathological examinations were performed on all animals. Tissues examined included the adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, lymph nodes, pancreas, parathyroids, pituitary gland, rectum, skin, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder. The mean body weights of male rats administered the high dose were slightly lower than the control animals throughout the study. The survival of male and female rats was similar between treated animals and controls (males: control 70/90 (78%); low dose 36/50 (72%); and high dose 38/50 (76%) and females: control 66/88 (75%); low dose 40/50 (80%) and high dose 37/50 (74%)). Histopathological examination revealed no evidence of carcinogenicity related to treatment with the test material. No other effects were reported. The authors reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of FD&C Yellow No. 6 in F344/N rats (NTP, 1981).

FD&C Red No. 40

In a Lifetime Toxicity/Carcinogenicity Study, FD&C Red No. 40 was provided in the diet as an admixture to Sprague-Dawley rats. In the *in utero* phase, 240 male and female rats were randomly assigned (30/group) to the control, low dose (0.37%), mid-

dose (1.39%) or high dose (5.19%) groups, providing daily intake levels of 180, 701 or 2,829 mg/kg bw/day for males and 228, 901 or 3,604 mg/kg bw/day for females. These parental (P_1) rats received the test material one week prior to mating, during the three-week mating period and during the gestation and lactation periods. The offspring of these animals were randomly selected and put into groups of fifty male and female weanling rats each. These groups were administered the test substance in the diet of the male animals for 118 weeks and the diet of female animals for 121 weeks at levels of 0, 0.37, 1.39 to 5.19 % corresponding to the dietary levels used in the *in utero* phase. Parameters included survival, clinical signs, body weight and food consumption, gross and microscopic pathology. Gross necropsies were performed on all animals dying during the study, all animals found in a moribund condition, and all animals killed at study termination. Complete histological examinations were performed on all animals in both the control and high-dose groups. The tissues examined histologically included: brain, pituitary, thoracic spinal cord, eyes, esophagus, thyroid, thymus, heart, lungs, liver, spleen, pancreas, stomach, small and large intestine, mesenteric lymph node, kidneys, adrenal, urinary bladder, uterus, prostate, ovaries, testes with epididymides, seminal vesicles, skin, rib junction, bone marrow, nerve with muscle, and any tissue masses or lesions. Histological examination was also performed on animals from any group with observable masses or lesions. If a potential effect was seen recurrently in a tissue, than that tissue was examined in all animals.

Food consumption was elevated among high dose males and females, but was not statistically significant. Red-tinted fur was reported among all treated animals, and red-tinted feces were reported for mid- and high-dose male and females. Group mean body weights of treated males and females were decreased compared to control animals at study termination, with the exception of mid-dose treated male rats that experienced an increase in mean body weight. However, the decrease in mean body weight was only statistically significant in female rats at the high dose level (3,604 mg/kg bw/day). Clinical chemistry and urinalysis parameters revealed no treatment related effects.

Histopathological examination revealed lesions in both control and treated animals at similar prevalence, and thus not attributed to test substance administration. No

biologically significant adverse effects were reported following administration of FD&C Red No. 40, with the exception of decrease mean body weights for high-dose female rats at study termination. The authors attributed this effect to the large amount of non-nutritive material in the diet at the intake level (Borzelleca *et al*., 1991a).

A similar lifetime/carcinogenicity study was also performed in Charles River HaM/ICR (CD-1) mice and in CD-1 outbred mice. In the *in utero* phase, 50 male and female CD-1 mice each (study A) or 70 male and female CD-1 outbred mice each (study B) were randomly assigned to the control, low dose (0.37%), mid-dose (1.39%) or high dose (5.19%) groups, providing daily intake levels of 507, 1,877 or 7,422 mg/kg bw/day for males and 577, 2,043 or 8,304 mg/kg bw/day for females (study A) and 492, 1,821, or 7,318 mg/kg bw/day (males) and 526, 2,057 or 8,356 mg/kg bw/day (females) (study B). These F_0 groups received the test material one week prior to mating, during the three week mating period and during gestation and lactation periods.

Groups of fifty male and female weanling CD-1 albino mice were randomly selected from the litters at 21 days of age and administered the FD&C Red No. 40 in the diet of study A animals for 104 weeks and the diet of study B animals for 109 weeks at levels of 0, 0.37, 1.39 or 5.19 %. These animals were the F_1 offspring of parental rats (F_0), which were treated at the corresponding levels. Study A had one control group while study B had two control groups. Parameters included survival, clinical signs, body weight and food consumption, gross and microscopic pathology. Gross necropsies were performed on all animals dying during the study, all animals found in a moribund condition, and all animals killed at study termination. Complete histology was conducted on all mice from all groups in study A and on 10/sex/group for the two control groups and the highest-dose group from study B. The tissues examined histologically included: brain, pituitary, thoracic spinal cord, eyes, esophagus, thyroid, thymus, heart, lungs, liver, spleen, pancreas, stomach, small and large intestine, mammary glands (study B only), mesenteric lymph node, kidneys, adrenal, urinary bladder, uterus, prostate, ovaries, testes with epididymides, seminal vesicles, skin, rib junction, bone marrow, nerve with muscle, and any tissue masses or lesions. Histological examination was also performed on

animals from any group with observable masses or lesions. If a potential effect was seen recurrently in a tissue, than that tissue was examined in all animals.

No treatment-related effects on survival were found. The authors reported decreased food consumption among the mid- and high-dose females for week 62-106 in study B. However, no consistent statistically significant effects on food consumption were reported in either study. Localized alopecia, labored respiration, colored hair coat, lacrimation and thinness were reported in similar incidences in both control and treated mice at all dose levels. Distended abdomens were noted in both mid- and high-dose females, while palpable masses were reported in control and treated groups at a similar incidence. Hematological and clinical chemistry parameters revealed few differences among treated and control groups. No significant gross pathological changes were reported among treated groups compared to control groups. An increase in absolute and relative thyroid weights in study B in the high-dose males and females was reported, but the significance was questioned because there was no accompanying histopathology, nor was it dose-dependent and it appeared to be species-specific.

The authors reported an earlier appearance of lymphatic lymphomas among treated groups in study A compared to control groups. No increases in incidence or appearance of lymphocytic lymphomas were reported in study B. The authors noted that study B was conducted using a different strain of mouse to further investigate if FD&C Red No. 40 had an effect on the appearance of lymphocytic lymphomas, and it revealed no relationship between the incidence of lymphocytic lymphomas and FD&C Red No. 40 (Borzelleca *et al.*, 1991b).

3.4.4 Developmental Toxicity

FD&C Red No. 40

Four groups of female Osborne-Mendel (FDA strain) rats (40-41 per group) were administered FD&C Red No. 40 in the drinking water at intake levels of 0, 0.2, 0.4 or 0.7% for the first 20 days of gestation. These intake levels correspond to daily doses of 0, 273.58, 545.68 or 939.29 mg/kg bw/day (Collins *et al*., 1989a). On day 20, the animals

were examined for gross abnormalities followed by euthanasia. Caesarean sections were performed. The uterus was examined for presence and position of resorption sites and fetuses, number of *corpora lutea* and implantation sites. All live fetuses were promptly weighed, sexed, and examined. Crown-rump lengths were measured. Fetuses were divided and assigned to skeletal or soft tissue examination. No clinical findings were reported and no deaths occurred during treatment. Mean fluid consumption was significantly increased in animals at the 0.2 and 0.4% intake levels, but only on days 14-20. Because fluid consumption was not increased at the 0.7% level, the findings were not considered significant. No other effects were reported.

A significant increase in the incidence of litters containing fetuses with missing sternebrae occurred in the 0.4% group, but not in the group receiving 0.7%. No dose related increases were reported for any sternebral variations. The number of fetuses with at least one type of sternebral variations was greater in all treated groups, but only significantly greater in the 0.4 and 0.7% groups. The percentage of total fetuses with at least one sternebral variation was greater in all of the treated groups compared to the control group, but the differences were not significant. The number of fetuses with more than one skeletal variation were similar among treated and control groups. The incidence of reduced ossification of the hyoid bone was significantly increased at the 0.7% intake level. Significant dose related increases were reported at the highest intake level for the average number of fetuses per litter with at least two skeletal variations and the number of litters containing them.

The authors questioned the biological significance of the reduced ossification of the hyoid bone given the lack of effect seen in a gavage study using higher dose levels. The increased incidence was slightly above that found in the historical controls, and the control group was noted as having a lower incidence compared to the historical controls (Collins *et al*., 1989a).

Four groups of female Osborne-Mendel (FDA strain) rats (42-43 per group) were administered FD & C Red No. 40 *via* gavage at dose levels of 0, 30, 75, 150, 300, 600 or 1000 mg/kg bw/day for the first 19 days of gestation. On day 19, the animals were

examined for gross abnormalities followed by euthanization. Caesarean sections were performed. The uterus was examined for presence and position of resorption sites and fetuses, number of corpora lutea and implantation sites. All live fetuses were promptly weighed, sexed, and examined. Crown-rump lengths were measured. Fetuses were divided and assigned to skeletal or soft tissue examination.

No clinical findings were reported and no deaths occurred during treatment. No other dose related findings were reported. The only significant skeletal anomaly found was an increase in 14th rib buds at the 300 mg/kg bw/day dose level but was not seen at the higher dose levels. No other soft-tissue or sternebral variations were reported. The NOAEL's for maternal and fetal toxicity were 1000 mg/kg bw/day (Collins *et al*., 1989b).

3.4.5 Reproductive Toxicity

FD&C Yellow No. 5

In a lifetime toxicity/carcinogenicity study, groups of rats (60/sex/group) were administered 0, 0, 0.1, 1.0 or 2.0% FD&C Yellow No. 5 in the diet daily for approximately 2 months prior to mating. In the high-dose study, 60/sex/group received 0 or 5% FD&C Yellow 5 for approximately 2 months prior to mating. The 3 controls groups received the basal diet only. A maximum of 2 rats/sex/litter were randomly selected for the chronic phase of the study. There were 70/sex/group at the initiation of the chronic phase and these offspring were exposed to the same dietary levels as their parents for 113 weeks. During the *in utero* phase, there were no treatment-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or number of live and still-born pups (Borzelleca and Hallagan, 1988a).

FD&C Yellow No. 6

In a three-generation reproduction study, 150 Charles River CD rats (10 males and 20 females/group/generation) received FD&C Yellow No. 6 at dietary levels of 0, 5, 50, 150, or 500 mg/kg/day. No treatment-related effects were observed in the parental rats or the pups receiving oral doses of up to 500 mg/kg bw/day (International Research and Development Corporation, 1974).

FD&C Red No. 40

Groups of male (10) and female (20) Charles River rats were administered FD&C Red No. 40 in the diet at 0, 3700, 13,900, or 51,900 ppm for 27 weeks prior to initiation of the first breeding phase. This P_1 parental generation was individually housed. Clinical observations included food consumption, appearance, individual body weights and behavior and were made weekly.

During the breeding phase of the P_1 generation, two females and one male were placed in a breeding cage. At weekly intervals during the mating period, the males were rotated among the females in each group. Following mating, the females were placed in individual cages to produce the first (FIA) litters. Twenty-four hours following the birth of the pups the first litters (FIA) were arbitrarily reduced to 8 maximum per mother. The number of conceptions, number of litters, live births, stillbirths, size of natural and nursing litters, deaths during the period of lactation, and number of pups weaned were recorded. The body weights of each pup were recorded at 24 hours and at weaning. Gross signs of toxicity were monitored. After 21 days of nursing, random pups were sacrificed and gross necropsies performed. Twenty-four females and twelve males remaining from each test group and control group were selected at random and designated the P_2 generation. Following the weaning of the F1A animals, the P_1 generation was remated to produce their second litters referred to as F1B, according to the procedures described above.

The P_2 generation was housed 4-5 per cage and was maintained on the same dietary levels as their parents. The procedures outlined above for the P_1 generation were

maintained for the P_2 generation. The litters of the P_2 animals were referred to as the F2A litters. Body weights of the F2A pups were monitored 24 hours following the birth and at weaning. Gross signs of toxicity were recorded. Following a 21 day nursing period, all pups were weaned and sacrificed. One week following the weaning period of the F2A litter, the P_2 generation was remated to produce their second litters (F2B). Two females were placed in a cage with a male from the corresponding dose group. Males were rotated weekly, and females were examined daily for presence of spermatozoa for a maximum of 21 consecutive days. The first day that sperm were observed was designated as day 0 of gestation. The females were then placed in individual cages. Half of the females (12) were sacrificed on day 19 or 20 of gestation and Caesarean sections were performed. Observations included number and placement of implantation sites, resorption sites, and live and dead fetuses, individual fetal weight and length (crown to rump), and external fetal anatomical structure. Gross necropsies were performed on each female including examination of uterus and visceral structures. The remaining 12 females were allowed to litter normally. The fetuses of both females delivering normally and *via* Caesarean section were necropsied.

Fertility indices for the control and test animals of both F1A and F1B were considered low. The authors attributed this to the advanced age of the animals upon mating. The fertility index of the 3,700 ppm test group in the F2A breeding cycle as well as the 3700 and 51,900 ppm test groups in the F2B breeding cycle were reported to be low in comparison to control animals and historical control data. Growth suppression, characterized as slight, was also reported for the low-level F1B pups, and the high-level F1A and F1B pups and the F2A and F2B breeding cycles when compared with controls. All other measured parameters were comparable to controls in each generation and among the two filial generations. The authors concluded that FD&C Red No. 40 caused meaningful growth suppression in the pups whose parents received the high level diets. The authors reported a no observable adverse effect level (NOAEL) for reproductive toxicity following administration of FD&C Red No. 40 as 13,900 ppm (Hazelton Laboratories, 1969).

3.4.6 New Testing Required

None.

3.5 TEST PLAN TABLE

Chemical	Physical-Chemical Properties				
	Melting Point	**Boiling Point**	**Vapor Pressure**	**Partition Coefficient**	**Water Solubility**
Sulfanilic acid CAS No. 121-57-3	A, Calc	Calc	Calc	A, Calc	A, Calc
o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid) CAS No. 6471-78-9	Calc	Calc	Calc	Calc	Calc

Chemical	Environmental Fate and Pathways			
	Photodegradation	**Stability in Water**	**Biodegradation**	**Fugacity**
Sulfanilic acid CAS No. 121-57-3	Calc	NA	A, Calc	Calc
o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid) CAS No. 6471-78-9	Calc	NA	R, Calc	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	**Acute Toxicity to Aquatic Invertebrates**	**Acute Toxicity to Aquatic Plants**
Sulfanilic acid CAS No. 121-57-3	A, Calc	A, Calc	R, Calc
o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid) CAS No. 6471-78-9	R, Calc	R, Calc	R, Calc

Chemical	Human Health Data					
	Acute Toxicity	**Genetic Toxicity *In Vitro***	**Genetic Toxicity *In Vivo***	**Repeat Dose Toxicity**	**Repro-ductive Toxicity**	**Develop-mental Toxicity**
Sulfanilic acid CAS No. 121-57-3	R	A	R	R	R	R
o-Toluene sulfonic acid, 4-amino-5-methoxy- (p-Cresidine sulfonic acid) CAS No. 6471-78-9	R	R	R	R	R	R

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties

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